WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.453
Volume 14, Issue 17, 661-681. Review Article ISSN 2277–7105

GUIDANCE ON STABILITY TESTING: STABILITY TESTING OF NEW DRUG SUBSTANCES AND PRODUCTS

[1*]Patel Nima Amaratbhai

[1*]Assistant Professor, Sharda School of Pharmacy, Pethapur.

Article Received on
13 July 2025,

Revised on 03 August 2025,
Accepted on 23 August 2025

DOI: 10.20959/wjpr202517-38177

***Corresponding Author**
Patel Nima Amaratbhai
Assistant Professor, Sharda School of Pharmacy, Pethapur.

ABSRACT

The purpose of the ICH Q1A revision is to enhance and clarify sections of the stability testing guideline. Key revisions include relocating the section on stress testing of the active substance from the glossary to the main text, aligning test procedures with ICH Q6A, introducing cross- references to other ICH guidelines, and amending testing frequency for accelerated conditions. Storage conditions are now more detailed, specifically addressing low temperature testing and aqueous liquids in semi-permeable containers. Post-approval commitments are clearly defined, and editorial inconsistencies, including glossary updates, have been corrected. Following the adoption of ICH Q1F, changes were made to storage conditions for Climatic Zones III and IV, notably updating intermediate storage to 30°C ± 2°C/65% RH ± 5% RH. Alternative long-term storage conditions were also introduced. Stability studies, governed by regulatory bodies like ICH, WHO, and FDA, are essential to ensure product safety, quality, and efficacy. This paper reviews global guidelines, types of stability studies, and their critical role in pharmaceutical development.

KEYWORDS: Stability studies, ICH Q1A(R), ICH Q1F, pharmaceutical products, stress testing, storage conditions, accelerated testing, semi-permeable containers, post-approval commitment, regulatory guidelines, shelf life, drug substance, drug product, WHO, FDA, CPMP, ICH guidelines.

INTRODUCTION

INTRODUCTORY NOTE TO REVISION OF NOVEMBER 2000

The purpose of the Q1A revision is to add information to certain sections and to provide clarification to other sections of the guideline.

The most important sections that have been revised are:

- The section on stress testing of the active substance has been moved from the glossary to the main text.
- The texts on test procedures etc. have been brought in line with the Q6A guideline. Relevant cross-references to other ICH guidelines have been introduced.
- The text on testing frequency has been amended for accelerated testing conditions.
- Storage conditions have been described in more detail. Testing at low temperature and testing of aqueous liquids in semi-permeable containers has been specifically addressed.
- The post-approval commitment is now described unambiguously

The document has also been revised to remove several editorial inconsistencies, including some revision of the Glossary.

INTRODUCTORY NOTE TO REVISION OF FEBRUARY 2003

The purpose of this note is to outline the changes made in Q1A(R) that result from adoption of ICH Q1F "Stability Data Package for Registration Applications in Climatic Zones III and IV". These changes are

1. The intermediate storage condition has been changed from 30°C ± 2°C/60% RH ± 5% RH to 30°C ± 2°C/65% RH ± 5% RH in the following sections:

- Drug Substance - Storage Conditions - General Case
- Drug Product - Storage Conditions - General Case
- Drug products packaged in semi-permeable containers
- Glossary - "Intermediate testing"

2. 30°C ± 2°C/65% RH ± 5% RH can be a suitable alternative long-term storage condition to 25°C ± 2°C/60% RH ± 5% in the following sections:

- Drug Substance - Storage Conditions - General Case
- Drug Product - Storage Conditions - General Case

3. 30°C ± 2°C/35% RH ± 5% RH has been added as a suitable alternative long-term storage condition to 25°C ± 2°C/40% RH ± 5% and the corresponding example for the ratio of water- loss rates has been included in the following section:

- Drug products packaged in semi-permeable containers

Mid-stream switch of the intermediate storage condition from 30°C ± 2°C/60% RH ± 5% RH to 30°C ± 2°C/65% RH ± 5% RH can be appropriate provided that the respective storage

conditions and the date of the switch are clearly documented and stated in the registration application.

It is recommended that registration applications contain data from complete studies at the intermediate storage condition 30°C ± 2°C/65% RH ± 5% RH, if applicable, by three years after the date of publication of this revised guideline.

Objectives of the Guideline

The following guideline is a revised version of the ICH Q1A guideline and defines the stability data package for a new drug substance or drug product that is sufficient for a registration application within the three regions of the EC, Japan, and the United States. It does not seek necessarily to cover the testing for registration in or export to other areas of the world.

The guideline seeks to exemplify the core stability data package for new drug substances and products, but leaves sufficient flexibility to encompass the variety of different practical situations that may be encountered due to specific scientific considerations and characteristics of the materials being evaluated. Alternative approaches can be used when there are scientifically justifiable reasons.

Scope of the Guideline

The guideline addresses the information to be submitted in registration applications for new molecular entities and associated drug products. This guideline does not currently seek to cover the information to be submitted for abbreviated or abridged applications, variations, clinical trial applications, etc.

Specific details of the sampling and testing for particular dosage forms in their proposed container closures are not covered in this guideline.

Further guidance on new dosage forms and on biotechnological/biological products can be found in ICH guidelines Q1C and Q5C, respectively.

General Principles

The purpose of stability testing is to provide evidence on how the quality of a drug substance or drug product varies with time under the influence of a variety of environmental factors such as temperature, humidity, and light, and to establish a re-test period for the drug substance or a shelf life for the drug product and recommended storage conditions.

The choice of test conditions defined in this guideline is based on an analysis of the effects of climatic conditions in the three regions of the EC, Japan and the United States. The mean kinetic temperature in any part of the world can be derived from climatic data, and the world can be divided into four climatic zones, I-IV. This guideline addresses climatic zones I and II. The principle has been established that stability information generated in any one of the three regions of the EC, Japan and the United States would be mutually acceptable to the other two regions, provided the information is consistent with this guideline and the labeling is in accord with national/regional requirements.

GUIDELINES FOR STABILITY STUDIES[18-40]

ICH and FDA Stability Regulatory Guidelines

Stability testing for product registration is one of the areas covered by international conference on harmonization (ICH) guidance documents. The ICH jointly governs the regulators and the industries involved in research from E U, United States, as well as Japan focusing on all its technical requirements for medicinal products containing new drugs. This organization was initiated in the early 1990s and stability testing was one of the first topics to proceed through the stepwise process to recognition by the regulatory bodies from all three region. Stability studies are currently an important method adopted within the manufacturing industries to develop brand new drug and new products. Stability study is often applied to suggest the conditions favourable to store the products and it highlights the fact that, the potency period or the expiry date of the drug has to be shown on the outer cover of packaging of the drugs for marketing, suggesting the drug to be safe and potent in its efficacy till its expiry date mentioned on the outer cover of the packaged product. are applied to suggested storage conditions and shelf life may be displayed on the label to confirm that the drug is safe and effective throughout its shelf life. Regulatory necessities are done more and more tight to attain the maximum goal in every potential condition to that the drug may well be placed throughout its shelf life. Hence the stability studies may be conducted after adopting good scientific principles, by properly knowing the present regulations governing the same and also keeping in mind the climatic zones.

Guidelines: A series of guideline documents were developed in order to clearly mention the stability information needed to register the new drug substances and products within the ICH regions. The stability studies performed to support product registration should comply with these guidance documents. Currently, there are five guidance documents available. They

begin with: **Q1A,** stability testing of new drug substances and products, which provides the basic protocol for stability studies for registration. For both new drug substances and new drug products, this guidance states the number and the types of batches, stability container closure systems, storage conditions, and time points that should be studied to support registration. It specifies that appropriate tests, analytical methods, and proposed acceptance criteria should be used, but references the ICH guidance documents on specifications and impurities for more detailed information. In addition to the general guidance on stability studies needed for registration, this document discusses stress testing of new drug substances and the required commitment to provide additionainformation on stability studies under taken on at least three production batches through the suggested retest time or till the expiry date, if not submitted in the original registration document. The remaining four documents supplement this general protocol guidance. There is guidance **Q1B,** photo stability testing of new drug substances and products.[2,6,8] This document provides instruction for carrying out photo stability studies on new drug substances and drug products to show that light exposure will not negatively impact the materials. The testing outlined is performed on one batch from the registration stability study and is a stepwise approach with exposed drug substance, exposed drug formulation, drug formulation soon after packaging, and the drug formulation packaged, for its distribution, as necessary. Guidance **Q1C,** stability testing of new dosage forms, was written to clarify the requirements for a new dosage form or line extension by the holder of the original submission. In this case, the requirements from Q1Aare followed, but less data may be required at the time of submission. In the parent guidance, Q1A, there is a mention of using bracketing or matrixing to reduce the amount of testing associated with the registration stability program. Guidance **Q1D**, bracketing and matrixing designs for stability testing of drug substances and drug products,[2,6,10] was written to provide more detailed guidance on the topic. This guidance discusses when each of these techniques may be considered and provides examples of them. It also discusses the potential risks with using these reduced testing designs. The fifth guidance, **Q1E**, evaluation of stability data, provides additional information relating to the method of evaluating and analysis of the information generated, statistically following the Q1A guideline. This document provides a stepwise process for evaluating stability data and extrapolating that information acquired to suggest the expiry date of the product. It discusses the application of linear regression, pool ability tests, and statistical modelling to stability data for registration. To supplement these guidance documents for the study of biotechnology products, an additional guidance was written. **Q5C,** quality of biotechnological products: stability testing of biotechnological/ biological product,

gives additional details for the stability testing of biotechnological/biological products for product registration. These guidance documents provide only the core requirements of the registration stability program. They do not provide all of the detail necessary to develop and manage the stability program in support of new product registration. Additionally, the abbreviated applications for registration of generic drug products is out of scope of the ICH documents but general principles may be taken from these guidance documents when studies to support registration are developed. In the past, the FDA provide additional stability guidance in a document issued in the year 1987, guideline for submitting documentation for the Stability of human drugs and biologics. This document was followed by a draft FDA guidance issued in the year 1998, guidance for industry: stability testing of drug substances and drug products. It combined the **ICH Q1AR2** with many different International Conference on Harmonization guidance's. This guidance became a basic referral guidance to all those carrying on studies on stability of drugs. International Conference on Harmonization issued Q1F guidance in the year 2004, which suggested stability study programmes carried on in order to support zone 3 and 4. Later on, the Association of South East Asian Nations (ASEAN) voiced regarding the conditions necessary for extremely hot and humid climate that are to be followed and implemented.[1] Both of the documents (the stability guidance passed in the year 1987, and the stability draft guidance passed in the year 1998), were withdrawn by the Food and Drug Association, in the year, June 2006. As a consequence, the ICH Q1F guideline withdrawn by the International Conference on Harmonization in July in the year 2006. As a part of the initiation taken by the agency, pharmaceutical current good manufacturing practices (cGMPs) for the 21st century came into being The QbD, quality-by-design concepts in drug development introduced by the Food and Drug Association became the most discussed topic of all times.

The names of the guidelines and their respective codes assigned to them by the International Conference on Harmonization guidance have been shown in the **Table 1**.

Table 1: Codes and Titles Used in ICH Guidelines.

ICH Code	Guideline title
Q1A	Stability testing of New Drug Substances and Products (Second Revision)
Q1A (R2)**2**	Stability testing of new drug substances and products**2**
Q1B	Stability testing: Photo stability testing of New Drug Substances and Products
Q1C	Stability testing of New Dosage Forms
Q1D	Bracketing and Matrixing Designs for stability testing of Drug Substances and Products

Q1E	Evaluation of stability data
Q1F	Stability data package for Registration Applications in Climatic Zones III and IV
Q5C	Stability testing of Biotechnological/Biological Products

Type of Stability of drug substance[1,4]

Physical Stability

The original physical properties, including appearance, palatability, uniformity, dissolution and suspend ability are retained. Physical stability affect to drug uniformity and release rate hence it is important from safety and efficiency point of view.

Chemical Stability

Each active ingredient retains its chemical integrity and labelled potency within the specified limits. The Chemical stability of drug is of great importance since it becomes less effective as it undergoes degradation. Also drug decomposition may yield toxic by-products that are harmful to the patient.

Microbiological Stability

Sterility or resistance to microbial growth is retained according to the Specified requirements. Antimicrobial agents retain effectiveness within specified limits. Microbiological instability of a sterile drug product could be hazardous.

Therapeutic Stability

The therapeutic effect remains unchanged.

Toxicological Stability

No significant increase in toxicity occurs.

GUIDELINES FOR STABILITY TESTING

To assure that optimally stable molecules and products are manufactured, distributed and given to the patients, the regulatory authorities in several countries have made provisions in the drug regulations for the submission of stability data by the manufacturers. Its basic purpose was to bring in uniformity in testing from manufacturer to manufacturer. These guidelines include basic issues related to stability, the stability data requirements for application dossier and the steps for their execution. Such guidelines were initially issued in 1980s.

These were later harmonized (made uniform) in the International Council for Harmonization (ICH) in order to overcome the bottleneck to market and register the products in other countries. The ICH was established in 1991, it was a consortium formed with inputs from both regulatory and industry from European commission, Japan and USA and various guidelines for drug substance and drug product came into existence regarding their quality, safety and efficacy. These guidelines are called as quality, safety, efficacy and multi-disciplinary (also called as Q, S, E and M) guidelines. 4 The World Health Organization (WHO), in 1996, modified the guidelines because the ICH guidelines did not address the extreme climatic conditions found in many countries and it only covered new drug substances and products and not the already established products that were in circulation in the WHO umbrella countries. In June 1997, United States Food and Drug Administration (US FDA) also issued a guidance document entitled 'Expiration dating of solid oral dosage form containing Iron. WHO, in 2004, also released guidelines for stability studies in global environment.7 ICH guidelines were also extended later for veterinary products.

A technical monograph on stability testing of drug substances and products existing in India has also been released by India Drug Manufacturers Association.8 Further, different test condition and requirements have been given in the guidance documents for active pharmaceutical ingredients, drug products or formulations and excipients. The codes and titles covered under ICH guidance have been outlined in the (Table 1) & (Table 2). Series of guidelines related to stability testing have also been issued by the Committee for Proprietary Medicinal Products (CPMP) under the European Agency for the Evaluation of Medicinal Products (EMEA) to assist those seeking marketing authorization for medicinal products in European Union.2 These are listed in (Table 3).

Table 2: ICH Q1A Summary of Stability Parameters.

Study Type & Condition		**Storage Condition**	**Time Period (Months)**	**Comments**
General Case:	**Long-term**	25 °C±2°C/60% RH±5% RH or 30°C±2°C/65% RH±5% RH	12	Must cover retest or shelf life period at a minimum and includes storage, shipment and subsequent use.
	Intermediate	30°C±2°C/65% RH±5% RH	6	
	Accelerated	40°C±2°C/75% RH±5% RH	6	
Refrigeration:	**Long-term**	5°C±3°C	12	Must cover retest or shelf life period at a minimum
	Accelerated	25°C±2°C/60%	6	

		RH±5% RH		and includes storage, shipment and subsequent use.
Freezer:	**Long term**	-20°C±5°C	12	Must cover shelf life period at a minimum and includes storage, shipment and subsequent use.

Table 3: CPMP Guidelines for Stability.

CPMP code	Guideline title
CPMP/QWP/576/ 96 Rev. 1	Guideline on Stability Testing for Applications for Variations to a Marketing Authorization
CPMP/QWP/6142/ 03	Guideline on Stability Testing for Active Substances and Medicinal Products Manufactured in Climatic Zones III and IV to be marketed in the EU
CPMP/QWP/609/ 96 Rev. 1	Note for guidance on Declaration of Storage Conditions for Medicinal Products Particulars and Active Substances
CPMP/QWP/122/0 2 Rev. 1	Note for Guidance on Stability Testing of Existing Active Substances and Related Finished Products
CPMP/QWP/072/96	Note for Guidance on Start of Shelf Life of the Finished Dosage Form
CPMP/QWP/2934/ 99	Note for Guidance for In-Use Stability Testing of Human Medicinal Products
CPMP/QWP/576/96	Note for Guidance on Stability Testing for a Type 2 variation to a Marketing Authorization
CPMP/QWP/ 159/96	Note for Guidance on Maximum Shelf-Life for Sterile Products after First Opening or Following Reconstitution

CLIMATIC ZONES FOR STABILITY TESTING

For the purpose of stability testing, the whole world has been divided into four zones (I - IV) depending upon the environmental conditions the pharmaceutical products are likely to be subjected to during their storage. These conditions have been derived on the basis of the mean annual temperature and relative humidity data in these regions. Based upon this data, long-term or real-time stability testing conditions and accelerated stability testing conditions have been derived. The standard climatic zones for use in pharmaceutical product stability studies have been presented in the (Table 4).[9] The break-up of the environmental conditions in each zone and also the derived long-term stability test storage conditions, as given by WHO have also been presented. The stability conditions have also been harmonized and adjusted to make them more practical for industry application and rugged for generalized application.

Table 4: ICH Climatic zones and long term stability conditions.

Climatic Zone	Climate Definition	Major Count ries /Region	MAT*/M ean annual partial water vapour pressure	Long-term testing conditions
I	Temperate	United Kingdom, Northern Europe, Russia, United states	<15C/<11hPa	21°C/45%RH
II	Subtropical and Mediterranean	Japan, Southern Europe	>15-22°C />11-18 hPa	25°C/60%RH
III	Hot and Dry	Iraq, India	>22°C/<15 hPa	30°C/35%RH
IVa	Hot and humid	Iran, Egypt	>22°C/>15-27 hPa	30°C/65%RH
IVb	Hot and very humid	Brazil, Singapore	>22°C/>27 hPa	30vC/75%RH

STABILITY TESTING PROTOCOL

Stability testing is the systematic approach towards drug development process. Stability data for the drug substance are used to determine optimal storage and packaging conditions for bulk lots of the material. The stability studies for the drug product are designed to determine the expiry date or shelf life.

The protocol for stability testing is a pre-requisite for starting stability testing and is necessarily a written document that describes the key components of a regulated and well-controlled stability study. Because the testing condition is based on inherent stability of the compound, the type of dosage form and the proposed container-closure system, the protocol depends on the type of drug substance or the product. In addition, the protocol can depend on whether the drug is new or is already in the market.[10,11] The protocol should also reflect the regions where the product is proposed to be marketed e.g. if the product is planned to be used in climatic zones I-III, IVa and IVb, the stability program must include all these zones.[11]

A well designed stability protocol should contain the following information:

- Number of Batches
- Containers and closures
- Orientation of storage of containers
- Sampling time points
- Test storage conditions
- Test parameters
- Test methodology
- Acceptance criteria

Numbers of Batches: Stability studies at developmental stages are generally carried out on a single batch while studies intended for registration of new product or unstable established product are done on first three production batches, while for stable and well-established batches, even two are allowed. If the initial data is not on a full scale production batch, first three batches of drug product manufactured post- approval should be placed on long-term studies using the same protocol as in approved drug application. Data on laboratory scale batches obtained during development of pharmaceuticals are not accepted as primary stability data but constitute supportive information. In general, the selection of batches should constitute a random sample from the population of pilot or production batches.[8]

Containers and Closures: The testing is done on the product in immediate containers and closures proposed for marketing. The packaging materials include aluminium strip packs, blister packs, Alu-Alu packs, HDPE bottles etc. This may also include secondary packs, but not shippers. Products in all different types of containers/closures, whether meant for distribution or for physician and promotional samples, are to be tested separately. However, for bulk containers, testing in prototype containers is allowed, if it simulates the actual packaging.

Orientation of Storage of Containers: Samples of the solutions, dispersed systems and semi- solid drug products for stability studies must be kept upright and positioned either inverted or on the side to allow for full interaction of the product with the container-closure. This orientation helps to determine whether the contact between the drug product or solvent and the closure results in the extraction of chemical substances from the closure components or adsorption of product components in to the container-closure.[10]

Sampling time points: Frequency of testing should be such that it is sufficient to establish the stability profile of the new drug substance. For products with a proposed shelf life of at least 12 months, the testing frequency at the long-term storage condition should be every 3 months over the first year, every 6 months over the second year and annually thereafter throughout the proposed shelf life expiration date. In the case of accelerated storage conditions, a minimum of three time points, including the initial and end points, for example, 0, 3, and 6 months is recommended. When testing at the intermediate storage condition is necessary as a result of significant change at the accelerated storage condition, a minimum of four test points, including the initial and final time points, is recommended, for example, 0, 6, 9 and 12 months.

In case the same product of different strengths, multiple sizes, etc is required to be tested, reduced stability testing plans can be worked out, which involves less number of test points. The reduced testing plans are based on bracketing and matrixing statistical designs. Bracketing is the design of a stability schedule such that only samples on the extremes of certain design factors, e.g., strength, package size, are tested at all-time points as in a full design. On the other hand, matrixing involves testing of a subset of the total number of possible samples for all combinations at a specific time point. Subsequently, another subset of samples for all factor combinations is tested. The factors that can be matrixes include batches, strengths with identical formulation, container sizes, fill sizes, and intermediate time points.[8,10]

Sampling Plan: Sampling plan for stability testing involves, planning for the number of samples to be charged to the stability chambers and sampling out of the charged batch so as to cover the entire study. The first step should be the development of the sampling time points followed by the number of samples needed to be drawn at each pull point for complete evaluation of all test parameters and finally adding up to get the total number of samples. For example there would be a requirement of about 100 tablets per pull out in a long term or accelerated stability studies including 10 each for assay, hardness and moisture determination, 6 each for dissolution and disintegration and 50 for friability. This multiplied by the total number of pull outs will give the total number of tablets required for a study. This is followed by the development of a sampling plan, which includes the selection of the containers representing the batch as a whole but in an unbiased manner. A stratification plan has been suggested whereby from a random starting point every n^{th} container is taken from the filling or packaging line (*n* is chosen such that the sample is spread over the whole batch).[8]

Test Storage Conditions: The storage conditions to be selected are based upon the climatic zone in which the product is intended to be marketed or for which the product is proposed to be filed for regulatory approval. General recommendations on the storage conditions have been given by ICH, CPMP and WHO. The abridged/indicative ICH and WHO storage conditions for drug products have been given in (Table 7).

Test Parameters*:* The stability test protocol should define the test parameters that would be used for evaluation of the stability samples. The tests that monitor the quality, purity, potency, and identity which could be expected to change upon storage are chosen as stability

tests. Therefore appearance, assay, degradation products, microbiological testing, dissolution, and moisture are standard tests performed on stability test samples. Microbiological tests include sterility, preservative efficacy and microbial count as applicable e.g. for liquid injectable preparations. The batches used for stability study must meet all the testing requirements including heavy metals, residue on ignition, residual solvents etc. Some of these are required at the time of product release but not required to be repeated during stability testing. Other tests like enantiomer purity, particle size and polymorphic form etc have also been discussed in ICH guidance Q6A.[11]

Test methodology: It is always recommended to follow the procedures given in the official compendia, as the results obtained using the official tests, in general find better acceptance. If alternate methods are used, they are required to be duly validated. However, the assay of the drug should be carried out using a stability-indicating method, established by carrying out stress tests on the drug under forced decomposition conditions. This method should be validated for specificity, accuracy, precision and linearity, in the range to which the drug is expected to fall during stability studies. For the assay of degradation products, the validated method should also include the limits of detection/quantification. The methods reported in literature should be used after confirming reproducibility and carrying out minimal validation, say of linearity, range, etc. It is always recommended to prepare a standard test protocol (STP) for each test.[8,10]

Acceptance criteria: All analytical methods are required to be validated before initiating the stability studies. Similarly, the acceptance criteria for the analytical results as well as that for the presence of degradation products should also be fixed beforehand. The acceptance criteria for each test in the stability study is fixed in the form of numerical limits for the results expressed in quantitative terms e.g., moisture pick-up, viscosity, particle size, assay, degradation products, etc. and pass or fail for qualitative tests e.g., odour, colour, appearance, cracking, microbial growth, etc. These acceptance criteria should also include individual and total upper limits for degradation products. ICH guideline Q3B(R2) related to impurities in new drug products addresses degradation products in new drug formulations. The degradation products of the active or interaction products from the active ingredients and excipients and/or active and container component should be reported, identified, and/or qualified when the proposed thresholds are exceeded. The reporting threshold of impurities is based upon the intended dose. If the maximum daily dose is less than or equal to 1gm, the

limit is 0.1% and if greater than 1, the limit is 0.05%. The identification threshold of impurities is between 1.0-0.1% for the maximum daily dose ranging between 1mg and 2gm.[8,11]

STABILITY TEST EQUIPMENT

The equipment used for stability testing is called stability chamber. These are specialized environmental chambers that can simulate the storage condition and enable evaluation of product stability based on real-time, accelerated and long-term protocols. They are available in both walk-in and reach-in styles. Smaller chambers are preferred for accelerated testing, as the retention time of products is much less in these cabinets, while the walk-in chambers are preferred for long-term testing. Such chambers or rooms are engineered and qualified to ensure uniform exposure of the set conditions to all the samples in the chamber. These chambers are expected to be dependable and rugged because of the requirement of uninterrupted use for years. They are fitted with appropriate recording, safety and alarm devices. In addition, photo stability chambers are also available and utilized both with and without temperature and humidity control. Two types of light sources are usually employed in photo stability chambers, one is the combination of cool white and near UV fluorescent tubes, while second are the artificial daylight lamps, e.g., xenon or metal halide. It is required to obtain a total exposure of 1.2 million lux h (h refers to hour). The visible light intensity is estimated using a lux meter. The calculation is made on how many hours of exposure are needed.[2,8]

STABILITY TESTING METHODS[2]

Stability testing is a routine procedure performed on drug substances and products and is employed at various stages of the product development. In early stages, accelerated stability testing (at relatively high temperatures and/or humidity) is used in order to determine the type of degradation products which may be found after long-term storage. Testing under less rigorous conditions i.e. those recommended for long-term shelf storage, at slightly elevated temperatures are used to determine a product's shelf life and expiration dates. The major aim of pharmaceutical stability testing is to provide reasonable assurance that the products will remain at an acceptable level of fitness/quality throughout the period during which they are in market place available for supply to the patients and will be fit for their consumption until the patient uses the last unit of the product.[12]

Depending upon the aim and steps followed, stability testing procedures have been categorized into the following four types.

- Real-Time Stability Testing
- Accelerated Stability Testing
- Retained Sample Stability Testing
- Cyclic Temperature Stress Testing

The major aim of pharmaceutical stability testing is to provide reasonable assurance that the products will remain at an acceptable level of fitness/quality throughout the period during which they are in market place available for supply to the patients and will be fit for their consumption until the patient uses the last unit of the product.

Real-Time Stability Testing: Real-time stability testing is normally performed for longer duration of the test period in order to allow significant product degradation under recommended storage conditions. The period of the test depends upon the stability of the product which should be long enough to indicate clearly that no measurable degradation occurs and must permit one to distinguish degradation from inter-assay variation. During the testing, data is collected at an appropriate frequency such that a trend analysis is able to distinguish instability from day-to-day ambiguity. The reliability of data interpretation can be increased by including a single batch of reference material for which stability characteristics have already been established. Stability of the reference material also includes the stability of reagents as well as consistency of the performance of the instrument to be used throughout the period of stability testing. However, system performance and control for drift and discontinuity resulting from changes in both reagents and instrumentation must be monitored.[2,13]

Accelerated Stability Testing: In accelerated stability testing, a product is stressed at several high (warmer than ambient) temperatures and the amount of heat input required to cause product failure is determined. This is done to subject the product to a condition that accelerates degradation. This information is then projected to predict shelf life or used to compare the relative stability of alternative formulations. This usually provides an early indication of the product shelf life and thus shortening the development schedule. In addition to temperature, stress conditions applied during accelerated stability testing are moisture, light, agitation, gravity, pH and package. In accelerated stability testing the samples are subjected to stress, refrigerated after stressing, and then assayed simultaneously. Because the

duration of the analysis is short, the likelihood of instability in the measurement system is reduced in comparison to the real-time stability testing. Further, in accelerated stability testing, comparison of the unstressed product with stressed material is made within the same assay and the stressed sample recovery is expressed as percent of unstressed sample recovery. For statistical reasons, the treatment in accelerated stability projections is recommended to be conducted at four different stress temperatures. However, for thermolabile and proteinaceous components, relatively accurate stability projections are obtained when denaturing stress temperatures are avoided.[13]

The concept of accelerated stability testing is based upon the Arrhenius equation (1) and modified Arrhenius equation (2).[13,14]

□ □ □= □ □ □+ Δ□ /RT(1)

where K = degradation rate/s,

A = frequency factor/s,

ΔE = activation energy (kJ/mol),

R = universal gas constant (0.00831 kJ/mol),

LOG K2 = $-Ea$ (1/T2.1/T1)

K12.303R

in the above equation (2) where k_1 and k_2 are rate constants at temperatures T_1 and T_2 expressed in degree kelvins; Ea is the activation energy; R is the gas constant.

These equations describe the relationship between storage temperatures and degradation rate. Using Arrhenius equation, projection of stability from the degradation rates observed at high Temperatures for some degradation processes can be determined. When the activation energy is known, the degradation rate at low temperatures may be projected from those observed at "stress" temperatures.[14,15,16] The stress tests used in the current ICH guideline (e.g., 40% for products to be stored at controlled room temperature) were developed from a model that assumes energy of activation of about 83 kJ per mole.[13]

A common practice of manufacturers in pharmaceutical industries was to utilize various shortcuts such as *Q* rule and bracket tables for prediction of shelf life of the products but these methods are not official either in ICH or FDA. The *Q* rule states that a product degradation

rate decreases by a constant factor *Q*10 when the storage temperature is decreased by 10°C.

The value of *Q*10 is typically set at 2, 3 or 4 because these correspond to reasonable activation energies. This model falsely assumes that the value of Q does not vary with temperature. The bracket table technique assumes that, for a given analyte, the activation energy is between two limits (e.g., between 10 and 20 kcal). As a result, a table may be constructed showing days of stress at various stress temperatures. The use of a 10 to 20 kcal bracket table is reasonable because broad experience indicates that most analytes and reagents of interest in pharmaceutical and clinical laboratories have activation energies in this range.[12,13]

Retained Sample Stability Testing*:* This is a usual practice for every marketed product for which stability data are required. In this study, stability samples, for retained storage for at least one batch a year are selected. If the number of batches marketed exceeds 50, stability samples from two batches are recommended to be taken. At the time of first introduction of the product in the market, the stability samples of every batch may be taken, which may be decreased to only 2% to 5% of marketed batches at a later stage. In this study, the stability samples are tested at predetermined intervals i.e. if a product has shelf life of 5 years, it is conventional to test samples at 3, 6, 9, 12, 18, 24, 36, 48, and 60 months. This conventional method of obtaining stability data on retained storage samples is known as constant interval method.[12,17] Stability testing by evaluation of market samples is a modified method which involves taking samples already in the market place and evaluating stability attributes. This type of testing is inherently more realistic since it challenges the product not just in the idealized retained sample storage conditions, but also in the actual marketplace.[12]

Cyclic Temperature Stress Testing*:* This is not a routine testing method for marketed products. In this method, cyclic temperature stress tests are designed on knowledge of the product so as to mimic likely conditions in market place storage. The period of cycle mostly considered is 24 h since the diurnal rhythm on earth is 24 h, which the marketed pharmaceuticals are most likely to experience during storage. The minimum and maximum temperatures for the cyclic stress testing is recommended to be selected on a product by-product basis and considering factors like recommended storage temperatures for the product and specific chemical and physical degradation properties of the products. It is also recommended that the test should normally have 20 cycles.[12,17]

CONCLUSION

Stability testing of pharmaceutical products the key procedural contribution in the development program for a new drug as well as new formulation. Any deviation from the established stability profile could affect the quality, safety and efficacy thorough understanding of the stability of the drug substance and drug product is important to "build the quality in". Stability tests are carried out so that recommended storage conditions and shelf life can be included on the label to ensure that the medicine is safe and effective throughout its shelf life. Over a period of time and with increasing experience and attention, the regulatory requirements have been made increasingly stringent to achieve the above goal in all possible conditions to which the product might be subjected during its shelf life. Therefore, the stability tests should be carried out following proper scientific principles and after understanding of the current regulatory requirements and as per the climatic zone.

REFERENCES

1. Thorat P, Warad S, Solunke R, Sargar A, Bhujbal A, Shinde A. Stability Study of Dosage Form: An Inovative Step, World Journal of Pharmacy and Pharmaceutical Sciences, **2014**; 3: 1031-1050.
2. Bajaj S, Singla D, Sakhuja N. Stability Testing of Pharmaceutical Products. Journal of Applied Pharmaceutical Science, **2012**; 2: 129-138.
3. ICH Q1A(R2). Stability testing guidelines: Stability testing of new drug substances and products. ICH Steering Committee, **2003;** 1-23.
4. Panda A, Kulkarni S., Tiwari R. Stability Studies: An Integral Part of Drug Development Process. International Journal of Pharmaceutical Research and Bio-Science, **2013**; 2: 69-80.
5. Valerie Sautou, Methodological Guidelines for Stability Studies of Hospital Pharmaceutical Preparations, Part 1: Liquid preparation. October, **2013**; 09-16.
6. Rao G., Goyal A., Development of Stability Indicating Studies for Pharmaceutical Products: An Innovative Step, International Journal of Pharmaceutical Chemistry and Analysis, **2016**; 3: 110-116.
7. WHO. Stability studies in a global environment. Geneva meeting working document QAS/05.146 with comments, **2004**.
8. Singh S., Bakshi M. Guidance on conduct of stress test to determine inherent stability of drugs. Pharmaceutical Technology Asia, Special Issue, Sep. /Oct, **2000**; 24-36.
9. Grimm W. Extension of the international conference on harmonization tripartite guideline

for stability testing of new drug substances and products to countries of climatic zones 3 and 4. Drug Development and Industrial Pharmacy, **1998**; 24: 313-325.

10. Ali J, Khar RK, Ahuja A. Dosage form and Design. Birla Publications Pvt. Ltd: Delhi, **2008**; 100-123.
11. Cha J, Gilmor T, Lane P, Ranweiler JS. Stability studies in Handbook of Modern Pharmaceutical Analysis. Separation Science and Technology. Elsevier, **2001**; 459-505.
12. Kommanaboyina B, Rhodes CT. Trends in stability Testing, with Emphasis on Stability During Distribution and Storage. Drug Development and Industrial Pharmacy, **1999**; 25: 857-867.
13. Anderson G, Scott M. Determination of product shelf life and activation energy for five drugs of abuse. Clinical Chemistry, **1991**; 37: 398-402.
14. Connors KA, Amidon GL, Kennon L. Chemical stability of pharmaceuticals-a handbook for pharmacists. John Wiley & Sons, New York, **1973**; 8-119.
15. Lachman L, Lieberman H. Kinetic principles and stability testing. The Theory and Practice of Industrial Pharmacy. CBS Publishers & distributors Pvt Ltd, New Delhi, Fourth Edition, **2013**; 1036-1072.
16. Bott RF, Oliveira WP. Storage conditions for stability testing of pharmaceuticals in hot and humid regions. Drug Development and Industrial Pharmacy, **2007**; 33: 393-401.
17. Carstensen JT, Rhodes CT. Rational Policies for Stability testing, Clinical Research and. Regulatory Affairs, **1993**; 10: 177-185.
18. Pharmaceutical Stability Testing to Support Global Market: Pharm asp, Edited by: Huynh Ba K, (2010) Published by: Springer Science+ Business Media, LLC, 2333 Spring Street, NY 10013, USA www.ICH.org.
19. Kaur M, Kaur H, Overview on Stability Studies. International Journal Pharmaceutical, Chemical and Biological Sciences, 2013; 3(4): 1231-1241.
20. Somvanshi Y and Satbhai P. ICH Guidelines and Main Focus on Stability Guidelines for New Formulation and Dosage Forms. World Journal of Pharmaceutical Research. 2015; 4(10): 561-578.
21. Chinchole AS, Poul BN, Panchal CV, and Chavan DV. A Review on Stability Guidelines by ICH and USFDA Guidelines for New Formulation and Dosage form. Pharma Tutor, 2014; 2(8): 32-53.
22. Eggert J & Flower C. Conducting Stability Studies during Development to Ensure Successful Regulatory Approval. In: Pharmaceutical Pre-Approval Inspections, a Guide to Regulatory Success. (2008) 2nd Edition. Edited by: Hynes III M D. Eli Lilly and

Company, Indianapolis, Indiana, USA. Published by: Informa Healthcare USA, Inc., 52 Vanderbilt Avenue, New York, NY 10017.

23. ICH. Harmonized tripartite guideline. Stability testing of new drug substances and Products Q1A (R2). February, 2003.
24. ICH. Harmonized Guideline: Stability Testing: Photo Stability Testing of New Drug Substances and Products Q1B, November, 1996.
25. ICH. Harmonized Guideline: Stability Testing: Requirements for New Dosage Forms QIC, November, 1996.
26. ICH. Harmonized Guideline: Bracketing and Matrixing Designs for Stability Testing of New Drug Substances and Products Q1D. February, 2002.
27. ICH. Harmonized Guideline: Evaluation of Stability Data Q1E, February, 2003.
28. ICH. Harmonized Guideline: Quality of Biotechnological Products: Stability Testing of Biotechnological/Biological Products Q5C, November, 1995.
29. FDA. Guidance for Industry: Guideline for Submitting Documentation for the Stability of Human Drugs and Biologics, 1987.
30. FDA. Draft Guidance for Industry: stability testing of drug substances and drug Products, 1998 (draft).
31. FDA. Pharmaceutical current good manufacturing practices (cGMP) for the 21st Century: a risk-based approach. Available at: www.fda.gov/cder/gmp.
32. FDA. Guidance for industry: quality systems approach to pharmaceutical CGMP Regulations, September 2006.
33. EMEA Committee for Proprietary Medicinal Products (CPMP), Guideline on Stability Testing: Stability Testing of Existing Active Substances and Related Finished Products CPMP/QWP/122/02, March 2004.
34. EMEA Committee for Medicinal Products for human Use (CHMP). Guideline on Stability Testing for Applications for Variations to a Marketing Authorization CPMP/QWP/576/96, December 2005.
35. EMEA CPMP. Note for Guidance on In-Use Stability Testing of Human Medicinal Products CPMP/QWP/2934/99, September2001 EMEA CPMP. Note for Guidance on Maximum shelf-life for sterile products for human use after first opening or following reconstitution. CPMP/QWP/159/96, July 1998.
36. EMEA CPMP. Note for Guidance on declaration of storage conditions: A: In the Product Information of Medicinal Products, B: For Active Substances. CPMP/QWP/609/96, October 2003.

37. EMEA CPMP. Note for Guidance on start of shelf-life of the finished dosage form CPMP/QWP/072/96, December 2001.
38. WHO. WHO Technical Report Series, No, 863, Annex 5 Guidelines for stability testing of pharmaceutical products containing well established drug substances in conventional dosage forms, 1996. Available at: www.who.int/medicines/areas/quality_safety/quality_assurance/regulator y_standards/en.
39. WHO. WHO Technical Report Series, No. 908. Item 11.1 WHO Guidelines for stability testing of pharmaceutical products containing well established drug substances in conventional dosage forms, 2003.
40. WHO. WHO Technical Report Series, No.937. Item 10.1 Stability Testing Conditions, 2006.